

SFB colonization

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 An abbreviated version of this protocol was published in Nature in Apr 2019

Compartmentalized gut lymph node drainage dictates adaptive immune responses

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Detailed protocol

1. fecal DNA extraction kit: Zymo research, Cat. No. D6010, use according to manufacturer's protocol
2. 16S primers:

SFB:

SFB736F: GACGCTGAGGCATGAGAGCAT

SFB844R: GACGGCACGGATTGTTATCA

UNIVERSAL(control):

UNIF340: ACTCCTACGGGAGGCAGCAGT

UNIR514: ATTACCGCGGCTGCTGGC

Program for q-PCR:

95C 3' (or 10 min if Applied Bio SYBR Green mix)

40 cycles of 10" at 95C and 45" at 62C.

per sample:

1 ul DNA

10 ul SYBR mix

0.4 ul primer mix at 20 nM each

8.6 ul water

The cycle at which SFB comes up depends on the machine. If you do not have germ free mice use a Jax mouse fecal pellet or, less ideal, antibiotics treated mouse fecal pellet as negative control. The universal 16S can be used as a reference/normalization (for non-antibiotic treated mice).

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Esterházy, D. and Mucida, D. (2021). SFB colonization. Bio-protocol Preprint. bio-protocol.org/prep1226.
2. Esterházy, D., Canesso, M. C., Mesin, L., Muller, P. A., Castro, T. B. D., Lockhart, A., ElJalby, M., Faria, A. M. and Mucida, D.(2019). Compartmentalized gut lymph node drainage dictates adaptive immune responses. Nature 569(7754). DOI: [10.1038/s41586-019-1125-3](https://doi.org/10.1038/s41586-019-1125-3)

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